

A Much better replacement of the Michaelis-Menten equation and its application

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May 19, 2015

Abstract

Michaelis-Menten equation is a basic equation of enzyme kinetics and gives an acceptable approximation of real chemical reaction processes. Analyzing the derivation of this equation yields the fact that its good performance of approximating real reaction processes is due to Michaelis-Menten curve (15). This curve is derived from Quasi-Steady-State Assumption(QSSA), which has been proved always true and called Quasi-Steady-State Law by Banghe Li et al [19].

Here, we found a quartic equation $A(S, E) = 0$ (22), which gives more accurate approximation of the reaction process in two aspects: during the quasi-steady state of a reaction, Michaelis-Menten curve approximates the reaction well, while our quartic equation $A(S, E) = 0$ gives better approximation; near the end of the reaction, our equation approaches the end of the reaction with a tangent line same to that of the reaction, while Michaelis-Menten curve does not. In addition, our quartic equation $A(S, E) = 0$ differs to Michaelis-Menten curve less than the order of $1/S^3$ as S approaches $+\infty$.

By considering the above merits of $A(S, E) = 0$, we suggest it as a replacement of Michaelis-Menten curve. Intuitively, this new equation is more complex and harder to understand. But, just because its complexity, it provides more information about the rate constants than Michaelis-Menten curve does.

Finally, we get a better replacement of the Michaelis-Menten equation by combing $A(S, E) = 0$ and the equation $dP/dt = k_2C(t)$.

keywords : rate constants of enzyme kinetics; quasi-steady-state assumption; quasi-steady-state law.

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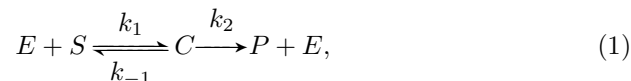
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1 Introduction

Enzymes are biological catalysts in almost all life processes. Enzyme kinetics as an important branch of enzymology studies the rate of reaction and the change of rate under different conditions. It is essential to describe the reaction mechanism[1].

In 1902, Adrian Brown studied the rate of hydrolysis of sucrose by yeast enzyme β -fructofuranosidase, which was considered as the first case study of enzyme kinetics[2]. Victor Henri proposed two reaction mechanisms which contains only one substrate and one product forming a substrate-enzyme complex[3, 4]. One of them became the basic model of enzyme kinetics:



where E , S , C , P represent enzyme, substrate, enzyme-substrate complex and product, respectively. And k_1 , k_{-1} , k_2 represent the rate constants of corresponding reaction steps.

Since Briggs and Haldane proposed the quasi-steady-state-assumption (QSSA) in 1925[5], this simplest model has been thoroughly studied under QSSA[1, 6, 7]. By QSSA, Briggs and Haldane obtained the classic Michaelis-Menten equation:

$$v_0 = V_{\max} S_0 / (K_M + S_0),$$

where v_0 is the initial velocity of the reaction, K_M is the Michaelis constant defined as $K_M = (k_{-1} + k_2) / k_1$ and V_{\max} is the so-called maximal velocity in many literatures, which is actually the supremum of the velocity but is never reached. Michaelis-Menten equation soon became the basic equation of enzyme kinetics[1]. All the experimental results so far show that Michaelis-Menten equation provides a good description of enzyme kinetics processes for large ensemble of

enzyme molecules when the concentration of substrate exceeds that of enzyme greatly. At the single-molecule level, the enzyme molecule moves according to thermal fluctuation and reacts stochastically with substrate molecules[8, 9]. By statistical analysis of the stochastic behaves, Michaelis-Menten equation also holds[10, 11].

After Briggs and Haldane’s work, Lineweaver and Burk[12] found that the reciprocal form of Michaelis-Menten equation gave a linear relation between $1/v_0$ and $1/S_0$, i. e.

$$1/v_0 = (K_M/V_{\max})1/S_0 + 1/V_{\max}. \quad (2)$$

This linear relation can be used to estimate the kinetics parameters with least square method. Although this estimation sometimes may lead to relative poor accuracy[13, 14, 15], many textbooks recognized its value on simplicity and visualization[1, 16, 17]. Michaelis-Menten equation do waste too much information on progress curve. In fact, Michaelis-Menten equation is derived from the quadratic equation $dE/dt = 0$ which can describe the whole process of the chemical reaction except the initial transient period provided $S_0 \gg E_0$ [18].

The validity of the Michaelis-Menten equation is strongly dependent on the validity of QSSA. Many biologists tested QSSA through biological experiments or computational experiments. But no one can confirm its validity during the next 80 years until recently Banghe Li et al. gave the rigorous description of this assumption and proved it mathematically[19]. Thus, from now on, this assumption is called the Quasi-Steady-State Law(QSSL). Moreover, this assures the validity of the Michaelis-Menten equation. This may be the first application of qualitative theory of dynamical systems into this basic enzyme kinetics model.

To quote the QSSL, we first introduce the basic model of enzyme kinetics. The enzyme kinetics is a branch of chemical kinetics[1]. Thus, according

to the law of mass action the time evolution of concentrations of reactants is determined by the following differential equations[20]:

$$dS/dt(t) = -k_1S(t)E(t) + k_{-1}C(t) \quad (3)$$

$$dE/dt(t) = -k_1S(t)E(t) + (k_{-1} + k_2)C(t) \quad (4)$$

$$dC/dt(t) = k_1S(t)E(t) - (k_{-1} + k_2)C(t) \quad (5)$$

$$dP/dt(t) = k_2C(t) \quad (6)$$

with the initial condition

$$(S(0), E(0), C(0), P(0)) = (S_0, E_0, 0, 0). \quad (7)$$

where $E(t)$, $S(t)$, $C(t)$ and $P(t)$ denote the concentrations of enzyme, substrate, enzyme-substrate complex and product at time t during the process, respectively. Under the two conservation laws

$$E(t) + C(t) = E_0 \quad (8)$$

$$S(t) + C(t) + P(t) = S_0, \quad (9)$$

these differential equations are equivalent to system of differential equations consisted of $(S(t), E(t))$, $(S(t), P(t))$ or $(P(t), E(t))$, i. e.

$$\begin{cases} dS/dt(t) = -k_1S(t)E(t) + k_{-1}(E_0 - E(t)) \\ dE/dt(t) = -k_1S(t)E(t) + (k_{-1} + k_2)(E_0 - E(t)) \end{cases}, \quad (10)$$

$$\begin{cases} dS/dt(t) = -k_1(S(t) + P(t) + E_0 - S_0)S + k_{-1}(S_0 - S(t) - P(t)) \\ dP/dt(t) = k_2(S_0 - S(t) - P(t)) \end{cases} \quad (11)$$

or

$$\begin{cases} dP/dt(t) = k_2(E_0 - E(t)) \\ dE/dt(t) = -k_1E(t)(S_0 - P(t) - E_0 + E(t)) + (k_{-1} + k_2)(E_0 - E(t)) \end{cases} \quad (12)$$

(10) is often used to analyze the basic model, but the other two forms are in fact equivalent to it, and sometimes are more convenient. These systems are nonlinear, and can not be integrated explicitly. However, they can be further simplified with the QSSL[19].

Quasi-Steady-State Law 1: Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $C(t)$ will go upwards from 0 at $t = 0$ to $E_0 - \varepsilon$ in a period less than ε , then it will stay in the interval between E_0 and $E_0 - \varepsilon$ until $S(t)/S_0 < \varepsilon$, if $S_0 > U$.

Quasi-Steady-State Law 2: Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $|dC/dt(t)|$ will be less than ε after a fast initial period less than ε and keep this state until $S(t)/S_0 < \varepsilon$, if $S_0 > U$.

Michaelis-Menten equation is derived from the quadratic equation $dE/dt = 0$, which is assured to be an acceptable approximate solution of the process after the initial transient period until S is nearly exhausted provided $S_0 \gg E_0$ by QSSLs. This article provides another equation which approximates the whole process of the chemical reaction better than $dE/dt = 0$ does. This replacement is first introduced in our former paper [21]. In [21], we provided an improved method to measure all rate constants in the simplest enzyme kinetics model using this replacement with the aid of Michaelis-Menten equation. This method improved the approach in [22] greatly. Here, we do deep analysis of this equation and found that all the three rate constants in the simplest enzyme kinetics model can be measured without Michaelis-Menten equation. The results are better than those gotten from using the Michaelis-Menten equation only, which shows

that this equation can replace the Michaelis-Menten equation.

The mathematical background can be found in many fundamental books on mathematical biology[23, 24] or ordinary differential equations[25, 26].

This article is organized as follows. Section 2 introduces the deviations of Michaelis-Menten curve and Michaelis-Menten equation which is not novel and can be read in many commentaries[27]. Section 3 gives our corresponding replacements of the curve and equation, and the merits for the replacements are given in section 4. Section 5 gives an application of the replacement of the Michaelis-Menten curve, and the conclusion comes in Section 6. Some subtle mathematics are left in Appendix.

2 Michaelis-Menten curve versus Michaelis-Menten equation

2.1 Derivation of Michaelis-Menten curve

Let t_1 be the time when the reaction attains its steady-state. According to QSSL2, after the initial transient, that is $t > t_1$, the reaction come to the steady-state:

$$dC/dt(t) \approx 0, \quad (13)$$

which is equivalent to

$$dE/dt(t) \approx 0. \quad (14)$$

Therefore, during the quasi-steady state of a reaction, the relationship about the concentrations $S(t)$ and $E(t)$ can be approximated by the following equation

$$0 = -k_1 S(t) E(t) + (k_{-1} + k_2)(E_0 - E(t)), \quad (15)$$

which yields

$$E_0 - E(t) = E_0 S(t)/(S(t) + K_M). \quad (16)$$

We name the curve of enzyme and substrate determined by the equation (15) or (16) as Michaelis-Menten curve.

2.2 Derivation of Michaelis-Menten equation

According to equation (6) and the Michaelis-Menten curve (16), we have

$$dP/dt(t) = k_2 E_0 S(t)/(K_M + S(t)), \quad (17)$$

or equivalently

$$v(t) = k_2 E_0 S(t)/(K_M + S(t)). \quad (18)$$

Let v_0 denote the initial velocity of the reaction, which is indeed the velocity when the reaction attains its steady-state, i. e. $dP/dt(t_1)$. Equation (17) becomes

$$v_0 = V_{\max} S(t_1)/(K_M + S(t_1)), \quad (19)$$

where $V_{\max} = k_2 E_0$. It may be assumed that

$$S(t) \approx S_0, \quad (20)$$

when $0 < t \leq t_1$ [20, 28] (A rigorous proof is given in Appendix). Therefore, the Michaelis-Menten equation is obtained

$$v_0 = V_{\max} S_0/(K_M + S_0). \quad (21)$$

Notice that, if v_0 is considered as a function of S_0 , v_0 is increasing and

$$\lim_{S_0 \rightarrow +\infty} v_0 = V_{\max}.$$

This is why biologists define $k_2 E_0$ as V_{\max} . They consider it as the maximal initial velocity. However, as we have shown, it can not be attained.

2.3 The determinant of Michaelis-Menten curve

By distinguishing Michaelis-Menten curve from Michaelis-Menten equation, we see clearly that the good performance of Michaelis-Menten equation approximating the real reactions is due to Michaelis-Menten curve.

Hence, if we find another curve which is a better approximation, then we can improve the classical Michaelis-Menten equation. Fortunately, we find one.

The following section gives our better replacements of Michaelis-Menten curve and Michaelis-Menten equation, respectively.

3 Replacements of Michaelis-Menten curve and Michaelis-Menten equation

For brevity here, we just give the formulas of the replacements of Michaelis-Menten curve and Michaelis-Menten equation, respectively. Their merits and motivations are given later.

3.1 Replacements of Michaelis-Menten curve

The replacement of Michaelis-Menten curve is

$$E(E_0 - E)[k_1SE - k_{-1}(E_0 - E)] + SE_0[k_1SE - (k_{-1} + k_2)(E_0 - E)] = 0. \quad (22)$$

We simply denote the left hand side of the above quartic equation as $A(S, E)$.

3.2 Replacements of Michaelis-Menten equation

Just like equation (16) represents an explicit solution $E(S)$ of equation (15), there is an explicit solution of equation (22) or $A(S, E) = 0$, too. Due to the complexity of the form, we denote $E(S) = x_2(S)$ here and give its detail in Appendix 7.3.

Hence, we get a replacement for Michaelis-Menten equation.

$$v_0 = k_2(E_0 - x_2(S_0)), \quad (23)$$

where $x_2 = -(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}/12a + (3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}) - b/(3a) + (1/2)\sqrt{3}i((36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}))$, $a = -k_1S - k_{-1}$, $b = (k_1S + 2k_{-1})E_0$, $c = -k_{-1}E_0^2 + (k_1S + k_{-1} + k_2)E_0S$ and $d = -(k_{-1} + k_2)E_0^2S$.

The detail form of equation (23) is somewhat complicated. However, for the purpose of applications, using the curve (22) instead of equation (23) is sufficient.

The following section will show that curve $A(S, E) = 0$ approximates real reactions better than Michaelis-Menten curve does, and hence the replacement of Michaelis-Menten equation is better than Michaelis-Menten equation.

4 Motivation and Derivation of the Replacement

The replacement of Michaelis-Menten curve was given first in [21]. In Section 4 of [21], we have given the motivation and derivation of the equation. For the convenience of the readers, we recap those here. For more information, please read [21].

This paper adopts the same notations. To be precisely, they are listed below again.

The first quadrant of the phase plane $S - E$ is divided into five regions as

$$\begin{aligned} L_1 &= \{(S, E) : Q(S, E) = 0, S \geq 0\}, \\ L_2 &= \{(S, E) : P(S, E) = 0, S \geq 0\}, \\ R_1 &= \{(S, E) : E > \tilde{E}, (S, \tilde{E}) \in L_1\}, \\ R_2 &= \{(S, E) : \tilde{E} > E > \hat{E}, (S, \tilde{E}) \in L_1, (S, \hat{E}) \in L_2\}, \\ R_3 &= \{(S, E) : E < \hat{E}, (S, \hat{E}) \in L_2\}. \end{aligned}$$

where

$$P(S, E) = -k_1SE + k_{-1}(E_0 - E), \quad (24)$$

$$Q(S, E) = -k_1SE + (k_{-1} + k_2)(E_0 - E). \quad (25)$$

The whole process of the reaction $(S(t), E(t))$ can be drawn on the $S - E$ plane. Since $S(t)$ decreases when t increases, we can consider E to be a function of S .

$$dE/dS = (-k_1SE(S) + (k_{-1} + k_2)(E_0 - E(S)))/(-k_1SE(S) + k_{-1}(E_0 - E(S))) \quad (26)$$

The solutions with its initial condition on the curve L_2 will vertically enter the region R_2 . Then, the concentration of substrate decreases and that of enzyme increases. In fact, these solutions will stay in R_2 forever and finally approaches the singular point. For sufficiently large initial concentration of substrate, these solutions go almost horizontally in R_2 , but at last they will approach the singular point with a certain slope. Therefore, there is an inflection point on each of these solutions.

We have

$$d^2E/dS^2 = k_1k_2A(S, E)/(k_1SE(S) - k_{-1}(E_0 - E(S)))^3. \quad (27)$$

Thus, the collection of inflection points satisfies $d^2E/dS^2 = 0$, that is $A(S, E) = 0$. As this system satisfies the existence and uniqueness condition of differential systems, any two different solutions will not intersect. Thus, the curve $A(S, E) = 0$ is just beneath the real process on the $S - E$ phase plane.

This is how we find the replacement $A(S, E) = 0$.

5 Reasons for the replacement being much better

We will give reasons that (22) is a much better replacement of Michaelis-Menten curve in this section.

In [21], we have observed that there is a part of $A(S, E) = 0$ lying in the region R_2 which approximates the real process well. It is denoted as L_3 . In fact L_3 is the replacement of Michaelis-Menten curve. Next, we will show that L_3 is a better approximation of real reaction than L_1 which is the Michaelis-Menten curve $Q(S, E) = 0$ (25). We only need to show that L_3 approximates

real reaction better than L_1 does.

5.1 Comparison in the major process of a reaction

When the reaction begins, S and E would decrease until they pass through the curve L_1 . In this period of the reaction, neither Michaelis-Menten equation nor its replacement L_3 can approximate the solution well.

Here, the major process of a reaction means that S and E are in the region of R_2 excluding the end of the reaction. The following two subsections will show that $A(S, E) = 0$ gives a more approximation of the real reaction processes than L_1 (Michaelis-Menten curve) by numerical instances under different conditions.

5.1.1 A case when QSSL condition violates

we choose $k_1 = 1$, $k_2 = 1$, $k_{-1} = 1$, $E_0 = 10$ but $S_0 = 20$. In this example the QSSL can not be used for S_0 is not sufficient large compared with E_0 . So L_1 may not be a good approximation of the solution. Before the reaction process approaches the region R_2 , both approximation of the solution are too bad. However, after the solution enters the region R_2 , L_3 gives a good approximation of the solution but L_1 doesn't, c. f. Fig 2.

5.1.2 Cases when QSSL condition holds

We denote $(S^*(t), E^*(t))$ to be the solution with initial condition that $(S^*(0), E^*(0)) = (S_0, E_0)$, $\hat{E}(S^*)$ to be the explicit form of approximate solution L_1 and $\tilde{E}(S^*)$ to be the explicit form of approximate solution L_3 . $E^*(t)$ is greater than $\tilde{E}(S^*(t))$ for all $t > 0$, which is proved in Appendix 6.1. $\hat{E}(S^*(t))$ is smaller than $E^*(t)$ when $t > \hat{t}$, which is proved by Lemma 3 in [19]. Here, \hat{t} is the time the real process touches the curve L_3 . That is to say, after the reaction enters the region R_2 , the real process lies between these two approximations. We choose $k_1 = 1$,

$k_2 = 1$, $k_{-1} = 1$, $E_0 = 10$ and $S_0 = 1000$ as the second example. In this example the QSSL can be used, so L_1 is a good approximation of the solution. During the reaction process, when $S^*(t) < 989.8$ or $t > 0.03$, the difference between $\tilde{E}(S^*(t))$ and $E^*(t)$ is less than the difference between $\hat{E}(S^*(t))$ and $E^*(t)$. That is to say L_3 is a better approximation of the solution after the initial transient period, i. e. less than 0.03.

We have also done another 250 numerical experiments. k_1 , k_2 and k_{-1} are chosen from $\{1, 3, 5, 7, 9\}$, and $E_0 = 0.5$, $S_0 = 20$ or 40. In each case, we divide $S(\hat{t})$ into 7 equal pieces with 6 point, which we denote from small to large as S_1, \dots, S_6 . We calculate the distances of E^* and \hat{E} and the distances of E^* and \tilde{E} . Table 1 shows the rate of these two numbers at the six points. These show that the curve L_3 approximate the solution better, and the smaller S is the better L_3 does.

5.2 Comparison near the ends of the reactions

Our new equation $A(S, E) = 0$, that is L_3 , approaches the end of reactions with a tangent line same to that of the reaction processes, while Michaelis-Menten curve does not. The following is the proof.

L_3 can be regarded as a graph of a function taking S as independent variable and E as dependent variable. The explicit form is given in Appendix 7.3. Rewrite $A(S, E) = 0$ as $(-k_1S - k_{-1})(E - E_0)^3 + (-2k_1SE_0 - k_{-1}E_0)(E - E_0)^2 + (SE_0k_{-1} - k_1E_0^2S + k_2E_0S + k_1S^2E_0)(E - E_0) + k_1S^2E_0^2 = 0$. Divide each side of the equation by S^2 and let $S \rightarrow 0$. Then,

$$k_{-1}(dE/dS(0))^2 + (k_1E_0 - (k_2 + k_{-1}))dE/dS(0) - k_1E_0 = 0. \quad (28)$$

Solving it, we get

$$dE/dS(0) = -\left(k_1 E_0 - (k_{-1} + k_2) + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}\right) / 2k_{-1} \quad (29)$$

where the other root is dropped for the slope must be negative. This slope is just the slope of the solution $(S^*(t), E^*(t))$ entering the point $(0, E_0)$. Thus, this part of $A(S, E) = 0$ give well approximation of $(S^*(t), E^*(t))$ even when $S(t)$ is very small. So, we confirm this part of $A(S, E)$ is a better approximation of a real reaction.

5.3 Comparison of the behaviors for large S

In [21], we saw that L_3 almost coincide with L_1 when S is sufficiently large. In fact, this can be proved. $A(S, E)$ equals to $E(E_0 - E)[k_1 S E - k_{-1}(E_0 - E)] + S E_0[k_1 S E - (k_{-1} + k_2)(E_0 - E)]$. Let (\hat{S}, \hat{E}) be the point on L_1 and (\hat{S}, \tilde{E}) on L_2 . Then, $A(\hat{S}, \hat{E}) = k_2 \hat{E}(E_0 - \hat{E})^2 > 0$ and $A(\hat{S}, \tilde{E}) = -k_2 \hat{S} E_0 (E_0 - \tilde{E}) < 0$. Thus, there must be one point E^* between \tilde{E} and \hat{E} such that $A(\hat{S}, E^*) = 0$. This proved that for each $S > 0$ there is a point of curve $A(S, E) = 0$ lies in R_2 . In fact, there is only one. The proof is given in Appendix 7.3.

Moreover, we can prove that, as $\hat{S} \rightarrow +\infty$, $(E^* - \hat{E})/(E^* - \tilde{E}) \rightarrow 0$. Since

$$0 = E^*(E_0 - E^*)[k_1 \hat{S} E^* - k_{-1}(E_0 - E^*)] + \hat{S} E_0[k_1 \hat{S} E^* - (k_{-1} + k_2)(E_0 - E^*)],$$

it can be proved that

$$(k_1 \hat{S} E^* - (k_{-1} + k_2)(E_0 - E^*)) / (k_1 \hat{S} E^* - k_{-1}(E_0 - E^*)) = -E^*(E_0 - E^*) / (\hat{S} E_0). \quad (30)$$

For

$$k_1 \hat{S} \hat{E} - (k_{-1} + k_2)(E_0 - \hat{E}) = 0, \quad (31)$$

$$k_1 \hat{S} \tilde{E} - k_{-1}(E_0 - \tilde{E}) = 0 \quad (32)$$

and

$$k_1 \hat{S} E^* - (k_{-1} + k_2)(E_0 - E^*) - (k_1 \hat{S} \hat{E} - (k_{-1} + k_2)(E_0 - \hat{E})) = (k_1 \hat{S} + k_{-1} + k_2)(E^* - \hat{E}),$$

$$k_1 \hat{S} E^* - k_{-1}(E_0 - E^*) - (k_1 \hat{S} \tilde{E} - k_{-1}(E_0 - \tilde{E})) = (k_1 \hat{S} + k_{-1})(E^* - \tilde{E}),$$

(30) can be written as

$$(k_1 \hat{S} + k_{-1} + k_2)(E^* - \hat{E}) / (k_1 \hat{S} + k_{-1})(E^* - \tilde{E}) = -E^*(E_0 - E^*) / (\hat{S} E_0). \quad (33)$$

Letting $\hat{S} \rightarrow +\infty$ on both side of (33),

$$(E^* - \hat{E}) / (E^* - \tilde{E}) \rightarrow 0$$

for $E^* \rightarrow 0$ when $\hat{S} \rightarrow +\infty$.

Thus, there is a part of $A(S, E) = 0$ in region R_2 asymptotically approaching to L_1 when S approaches $+\infty$.

According to (33),

$$E^* - \hat{E} = -E^*(E_0 - E^*)(k_1 \hat{S} + k_{-1})(E^* - \tilde{E}) / (\hat{S} E_0 (k_1 \hat{S} + k_{-1} + k_2)). \quad (34)$$

Note that $E^* \leq \hat{E}$, $E_0 - E^* \leq E_0$ and $E^* - \tilde{E} \leq \hat{E} - \tilde{E}$. Because of (31), $\lim_{\hat{S} \rightarrow +\infty} \hat{E} \hat{S} = K_M$. The difference of (31) and (32) is

$$k_1 \hat{S}(\hat{E} - \tilde{E}) - (k_{-1} + k_2)(E_0 - \hat{E}) + k_{-1}(E_0 - \tilde{E}) = 0.$$

$\hat{S} \rightarrow +\infty$ implies $\hat{S}(\hat{E} - \tilde{E}) \rightarrow k_2 E_0 / k_1$. These together with (34) yields $E^* - \hat{E} = O(1/\hat{S}^3)$.

6 Application

The above section has shown that the curve $A(S, E) = 0$ approximates the trajectory $(S(t), E(t))$ of the reaction (1) better than the curve $Q(S, E) = 0$ does. In this section, we will show that $A(S, E) = 0$ not only gives more information about the relationships among the three rate constants but also gives more accurate evaluations of these constants.

6.1 $A(S, E) = 0$ gives more information about the rate constants

For convenience, we repeat $A(S, E)$ and $Q(S, E)$ here again as

$$\begin{aligned} A(S, E) &= E(E_0 - E)[k_1SE - k_{-1}(E_0 - E)] + SE_0[k_1SE - (k_{-1} + k_2)(E_0 - E)], \\ Q(S, E) &= -k_1SE + (k_{-1} + k_2)(E_0 - E). \end{aligned}$$

Rearranging the items of the right side of $A(S, E)$ yields that

$$\begin{aligned} A(S, E) &= k_1(-SE^3 + E_0SE^2 + E_0S^2E) \\ &\quad + k_2(E_0SE - E_0^2S) \\ &\quad + k_{-1}(-E^3 + 2E_0E^2 + E_0SE - E_0^2E - E_0^2S). \end{aligned}$$

By comparing equations $A(S, E)$ and $Q(S, E)$, we find that given some values of (S, E) , we can calculate all the three values of k_1 , k_2 and k_{-1} up to a common multiplier by equation $A(S, E) = 0$, but we only get two values of k_1 and $(k_{-1} + k_2)$ up to a common multiplier by equation $Q(S, E) = 0$. In other words, $Q(S, E)$ only contains the information about the Michaelis constant K_M as a whole, but $A(S, E)$ contains the information of $a = k_2/k_1$ and $b = k_{-1}/k_1$, which also yield Michaelis constant by $K_M = a + b$.

Moreover, by including an additional equation

$$dP/dt = k_2(E_0 - E). \quad (35)$$

This is just (6), from which k_2 can be measured, $Q(S, E) = 0$ only provides the information about K_M and k_2 , while $A(S, E) = 0$ provides that of all the three rate constants k_1 , k_2 and k_{-1} .

Since equations (6) and $Q(S, E) = 0$ consist of the origin of Michaelis-Menten equation, the compounding of equations (6) and $A(S, E) = 0$ gives more information about the rate constants than Michaelis-Menten equation does.

6.2 $A(S, E) = 0$ gives more accurate evaluations of the rate constant

As an example, we design a numerical experiment to show that compared with $Q(S, E) = 0$, $A(S, E) = 0$ not only gives more information about the relationships among the three rate constants but also gives more accurate evaluations of these constants. In the example, we set the rate constants as $k_1 = 0.3$, $k_2 = 0.2$ and $k_{-1} = 0.1$, and the initial concentrations of enzyme and substrate as $E_0 = 0.5$ and $S_0 = 20$. Some points of (S, E) are measured on the trajectory of the reaction, and then the results are calculated by $Q(S, E) = 0$ and $A(S, E) = 0$, respectively. All the results are listed in Table (2).

In table (2), we only list the concentrations of substrate, and do not list the corresponding concentrations of enzyme for brevity. $a : h : b$ means that the concentrations of substrate are measured from $S = a$ to $S = b$ with a step length h . After measured these values of S and their corresponding values of E , K_M can be calculated by $Q(S, E) = 0$ and $A(S, E) = 0$, respectively.

For different sets of points (S, E) as chosen in table (2), K_M^A is always closer to its exact value 1 than K_M^Q . It is even the case, when there are only two points

in the set, such as $(S = 3, E = 0.1234)$ and $(S = 19, E = 0.0250)$.

Another phenomena observed from this table is that for both equations $Q(S, E) = 0$ and $A(S, E) = 0$, K_M is more accurate when the data set is measured closer to the core region of the steady state of the reaction. Such a phenomena also gives another support that both $Q(S, E) = 0$ and $A(S, E) = 0$ approximate the real reaction well at the quasi-steady state, moreover $A(S, E) = 0$ is better than $Q(S, E) = 0$.

If k_2 is measured by equation (6), then k_1 and k_{-1} are all known due to equation $A(S, E)$. In this example, we assume that $k_2 = 0.200$, and hence, the estimated values of k_1 and k_{-1} are listed in the table, too.

Now, we have completely shown that compared with $Q(S, E) = 0$, $A(S, E) = 0$ not only gives more information about the relationships among the three rate constants but also gives more accurate evaluations of these constants. Thus, we claim that $A(S, E) = 0$ is a better replacement of Michaelis-Menten curve, and combined with (6) gives a better replacement of Michaelis-Menten equation.

7 Conclusion

In this article, we propose another curve that can replace the Michaelis-Menten curve and another equation that can replace the Michaelis-Menten equation. We used this new curve to estimate all the rate constants of the basic enzyme kinetics model. Results show that this replacement does very well. The Michaelis-Menten curve only gives information about K_M . The Michaelis-Menten equation, which is derived by combining Michaelis-Menten curve and (6), only gives information about K_M and k_2 . By contrasting to Michaelis-Menten curve, the replacement curve gives more information. And then, the replacement equation gives information about k_1 , k_{-1} and k_2 . Numerical experiments show that these replacements not only give more information about the relationships among the

three rate constants but also give more accurate evaluations of these constants.

We did not give the mathematical meaning and reasoning that the replacement curve gives better approximate than Michaelis-Menten curve during the major process. Instead, we only give some numerical examples. We hope to do so in future work.

8 Appendix

8.1 $S(t)$ in the initial transient period of a reaction

To obtain the Michaelis-Menten equation, (20) is assumed in former literatures. Here, we prove it under the conditions in QSSLs. That is to say if S_0 is much more larger than E_0 , $S(t)$ is nearly equal to S_0 when $0 < t \leq t_1$. To be more precise and rigorous, we state it as a lemma below.

Lemma: Given E_0 and any small positive number $\varepsilon > 0$, there is a proper positive number U such that $|dC/dt(t)|$ will be less than ε after a fast initial period t_1 less than ε , and keep this state until $S(t)/S_0 < \varepsilon$, if $S_0 > U$. Moreover, $|S(t)/S_0| \geq 1 - \varepsilon$, for $0 < t \leq t_1$.

Proof: The first part of the theorem is just the QSSL2. According to Lemma 3 in [19], $dS/dt(t) < 0$ for $t > 0$. For equation (3),

$$\begin{aligned}
 |dS/dt(t)| &= |-k_1S(t)E(t) + k_{-1}C(t)| \\
 &= k_1S(t)E(t) - k_{-1}C(t) \\
 &\leq k_1S(t)E(t) \\
 &\leq k_1S_0E_0.
 \end{aligned} \tag{36}$$

Because of QSSL2, we could find U_1 and U_2 satisfies the following two statements, respectively. Given E_0 and any small positive number $\varepsilon/(k_1E_0) > 0$, there is a proper positive number U_1 such that $|dC/dt(t)|$ will be less than

$\varepsilon/k_1 E_0 > 0$ after a fast initial period less than $\varepsilon/k_1 E_0 > 0$, and keep this state until $S(t)/S_0 < \varepsilon/k_1 E_0 > 0$, if $S_0 > U_1$. Given any small positive number $\varepsilon > 0$, there is a proper positive number U_2 such that $|dC/dt(t)|$ will be less than ε after a fast initial period less than ε and keep this state until $S(t)/S_0 < \varepsilon$, if $S_0 > U_2$. Choose U such that $U > U_1$ and $U > U_2$. Then, if $S_0 > U$, the first statement of the theorem is proved. Moreover, for (36)

$$S(t)/S_0 \geq (S_0 - \varepsilon k_1 S_0 E_0 / (k_1 E_0)) / S_0 = 1 - \varepsilon. \quad (37)$$

when $0 < t \leq t_1$. This completes the proof. \square

Now

$$v_0 = V_{\max} S(t_1) / (K_M + S(t_1)) = V_{\max} S_0 / (K_M S_0 / S(t_1) + S_0) \approx V_{\max} S_0 / (K_M + S_0),$$

because $1 \leq S_0 / S(t_1) \leq 1 / (1 - \varepsilon)$, and ε can be arbitrarily small.

8.2 The convexity of $(S^*(t), E^*(t))$

The solution $(S^*(t), E^*(t))$ do not have any inflection point at all, i. e. $(S^*(t), E^*(t))$ do not go across $A(S, E) = 0$ and $(S^*(t), E^*(t))$ lies above L_3 . Assume $(S(t), E(t))$ is a solution of system (10), and at time t_2 it intersects with $A(S, E) = 0$ at $(S(t_2), E(t_2))$. Consider $A(S(t), E(t)) = E(t)(E_0 - E(t))[k_1 S(t)E(t) - k_{-1}(E_0 - E(t))] + S(t)E_0[k_1 S(t)E(t) - (k_{-1} + k_2)(E_0 - E(t))]$. Differentiate it with respect to t , and note that $dS/dt = P(S, E)$, $dE/dt = Q(S, E)$:

$$dA/dt = -Q(E_0 - E)P + EQP - E(E_0 - E)dP/dt - PQE_0 - SE_0dQ/dt. \quad (38)$$

For simplicity, we write A as $A(S(t), E(t))$, P as $P(S(t), E(t))$, Q as $Q(S(t), E(t))$, E as $E(t)$ and S as $S(t)$. Simple calculation shows that

$$dP/dt = -k_1PE - k_1SQ - k_{-1}Q \quad (39)$$

and

$$dQ/dt = -k_1PE - k_1SQ - k_{-1}Q - k_2Q. \quad (40)$$

As $A(S(t_2), E(t_2)) = 0$,

$$SQE_0 = -E(E_0 - E)P \quad (41)$$

at point $t = t_2$. By putting (39), (40) and (41) in (38),

$$dA/dt(t_2) = -2P(S(t_2), E(t_2))Q(S(t_2), E(t_2))(E_0 - E(t_2)). \quad (42)$$

For (??), $dA/dt(t_2) > 0$. Therefore, if the solution $(S(t), E(t))$ of (10) has one point t_0 in the region R_2 satisfying $A(S(t_0), E(t_0)) > 0$, then $A(S(t), E(t)) \geq 0$ for $t > t_0$. We have proved that $A(\hat{S}, \hat{E}) = k_2\hat{E}(E_0 - \hat{E})^2 > 0$, where (\hat{S}, \hat{E}) is on L_1 . Assume at time t_4 , $(S^*(t), E^*(t))$ reached the curve L_1 . Thus, $A(S^*(t_4), E^*(t_4)) > 0$. For continuity, there is a ε , such that for any $t_4 + \varepsilon > t_5 > t_4$, $(S(t_5), E(t_5))$ is in the region R_2 and $A(S^*(t_5), E^*(t_5)) > 0$. Then, $A(S^*(t), E^*(t)) > 0$ for $t \geq t_4$. Moreover, $A(S^*(t), E^*(t)) > 0$ for $0 < t < t_4$, i. e. $(S(t), E(t)) \in R_1$, can be verified by straight calculation. According to (27), $(S^*(t), E^*(t))$ is convex.

8.3 The explicit form

In this subsection, we talk about the explicit form of the curve L_3 . $A(S, E) = 0$ is a three degree equation of E . Thus, for each $S > 0$, there are at most

three real solutions of E . We have proved that there is at least one solution of E in the region R_2 for any $S > 0$. As in Section 3.2, we have proved that $A(\hat{S}, \hat{E}) = k_2 \hat{E}(E_0 - \hat{E})^2 > 0$ and $A(\hat{S}, \tilde{E}) = -k_2 S E_0 (E_0 - E) < 0$. Note that,

$$\lim_{E \rightarrow \infty} A(\hat{S}, E)/E^3 = -k_1 \hat{S} - k_{-1} \quad (43)$$

for any $\hat{S} > 0$. Thus, when E is positively sufficiently large, $A(\hat{S}, E) < 0$. And when E is negatively sufficiently large, $A(\hat{S}, E) > 0$. For the continuity of $A(S, E)$, there is at least one real solution greater than \hat{E} and there is at least one real solution less than \tilde{E} . We have already found three solutions of E when $S > 0$, so there are exact three solutions of E when $S > 0$. The explicit form of all these three solutions, denoted by $E = x_1(S)$, $E = x_2(S)$ and $E = x_3(S)$, can be given in mathematics.

The three solutions of the equation $ax^3 + bx^2 + cx + d = 0$ are

$$x_1 = (36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}) - b/(3a),$$

$$x_2 = -(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}/12a + (3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}) - b/(3a) + (1/2)\sqrt{3}i((36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}))$$

and

$$x_3 = -(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}/12a + (3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}) - b/(3a) - (1/2)\sqrt{3}i((36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3}/6a - 2(3ac - b^2)/(3a(36abc - 108a^2d - 8b^3 + 12\sqrt{3}(4ac^3 - b^2c^2 - 18abcd + 27a^2d^2 + 4b^3d)^{1/2}a)^{1/3})).$$

In this problem, $a = -k_1S - k_{-1}$, $b = (k_1S + 2k_{-1})E_0$, $c = -k_{-1}E_0^2 + (k_1S + k_{-1} + k_2)E_0S$ and $d = -(k_{-1} + k_2)E_0^2S$.

We have proved that all the three solutions are real, so we should decide which one represent the curve L_3 .

We choose $k_1 = 1$, $k_2 = 1$, $k_{-1} = 1$, $E_0 = 1$ and $S = 1$. Then, $x_1 \approx -0.8892$, $x_2 \approx 0.6446$ and $x_3 \approx 1.7446$. Therefore, x_2 is the right one in the region R_2 . Because x_1 , x_2 and x_3 are continuous functions of k_1 , k_2 , k_{-1} , E_0 and S , and x_1 , x_2 and x_3 can not coincide for any $k_1 > 0$, $k_2 > 0$, $k_{-1} > 0$, $E_0 > 0$ and $S > 0$, we can conclude that $E = x_2(S)$ is the explicit form of L_3 , i. e. the replacement of the Michaelis-Menten equation.

Acknowledgments

This work is partially supported by a National Key Basic Research Project of China (2011CB302400), by National Natural Science Foundation of China (11301518) and by the National Center for Mathematics and Interdisciplinary Sciences, CAS.

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Figure 1: The S - E phase plane.

Figure 2: L_3 gives a good approximation of the solution after the solution enters the region R_2 .

Table 1: 250 numerical experiments.

Table 2: Rate constants estimated by $A(S, E) = 0$ or $Q(S, E) = 0$. The first column indicates the measured concentrations of the substrate during the reaction process, and the corresponding concentrations of enzyme is determined by S , so we do not show them explicitly. $a : h : b$ means that the concentrations are measured from $S = a$ to $S = b$ with step length h . K_M^Q denotes the Michaelis constant K_M estimated by $Q(S, E) = 0$, K_M^A denotes the K_M estimated by $A(S, E) = 0$, k_1^A and k_{-1}^A denote k_1 and k_{-1} estimated by $A(S, E) = 0$, if k_2 is provided. Here, we assume that $k_2 = 0.2000$ is exactly estimated by equation (35).

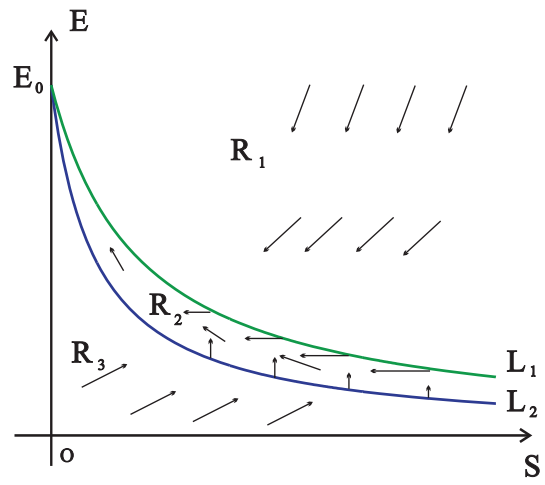


Figure 1: The S - E phase plane.

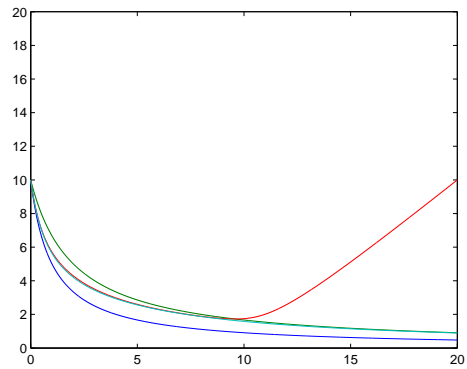


Figure 2: L_3 gives a good approximation of the solution after the solution enters the region R_2

k_1	k_2	k_{-1}	S_0	E_0	S_1	S_2	S_3	S_4	S_5	S_6
1	1	1	40	0.5	1360.849	978.494	659.542	404.013	211.969	83.724
1	1	3	40	0.5	1607.115	1190.643	837.895	549.093	324.898	168.645
1	1	5	40	0.5	1880.447	1430.567	1045.033	724.584	471.446	296.762
1	1	7	40	0.5	2182.374	1700.043	1283.124	933.340	655.858	476.536
1	1	9	40	0.5	2514.348	2000.800	1554.320	1178.212	882.388	716.419
1	3	1	40	0.5	532.045	393.568	276.394	180.568	106.235	54.160
1	3	3	40	0.5	623.350	473.711	345.536	239.036	154.855	96.309
1	3	5	40	0.5	724.132	563.671	424.992	308.626	216.180	155.887
1	3	7	40	0.5	834.896	664.039	515.482	390.276	291.599	235.622
1	3	9	40	0.5	956.131	775.389	617.712	484.918	382.495	338.216
1	5	1	40	0.5	372.294	282.761	206.082	142.360	91.916	56.441
1	5	3	40	0.5	432.776	336.705	253.673	183.969	128.482	91.841
1	5	5	40	0.5	499.232	396.886	307.883	232.825	173.536	139.448
1	5	7	40	0.5	571.961	463.651	369.141	289.489	227.906	200.886
1	5	9	40	0.5	651.252	537.344	437.867	354.516	292.417	277.761
1	7	1	40	0.5	308.005	239.517	180.320	130.602	90.937	64.393
1	7	3	40	0.5	355.468	282.472	218.981	165.402	122.974	98.218
1	7	5	40	0.5	407.406	330.128	262.678	205.787	161.686	142.000
1	7	7	40	0.5	464.028	382.732	311.712	252.155	207.663	196.886
...
7	5	9	40	0.5	1905.853	1368.871	921.202	562.884	294.032	115.236
7	7	1	40	0.5	1261.696	893.181	588.432	347.446	170.216	56.703
7	7	3	40	0.5	1293.832	920.402	610.742	364.855	182.747	64.456
7	7	5	40	0.5	1326.463	948.129	633.572	382.803	195.852	72.877
7	7	7	40	0.5	1359.591	976.365	656.927	401.299	209.543	81.987
7	7	9	40	0.5	1393.227	1005.120	680.817	420.352	223.832	91.812
7	9	1	40	0.5	1004.972	714.795	474.213	283.225	141.824	49.985
7	9	3	40	0.5	1030.374	736.374	491.974	297.178	151.996	56.486
7	9	5	40	0.5	1056.165	758.350	510.144	311.557	162.624	63.526
7	9	7	40	0.5	1082.346	780.727	528.727	326.368	173.717	71.122
...
9	5	7	20	0.5	632.410	458.999	313.794	196.824	108.175	48.333
9	5	9	20	0.5	657.240	480.424	331.837	211.529	119.657	57.071
9	7	1	20	0.5	416.007	297.340	198.796	120.367	62.035	23.724
9	7	3	20	0.5	432.973	311.831	210.819	129.935	69.181	28.595
9	7	5	20	0.5	450.325	326.725	223.264	139.952	76.824	34.092
9	7	7	20	0.5	468.080	342.036	236.146	150.433	84.981	40.245
9	7	9	20	0.5	486.247	357.774	249.473	161.389	93.667	47.083
9	9	1	20	0.5	335.698	241.688	163.326	100.608	53.515	21.984
9	9	3	20	0.5	349.216	253.282	173.005	108.384	59.425	26.189
9	9	5	20	0.5	363.046	265.201	183.022	116.521	65.737	30.907
9	9	7	20	0.5	377.191	277.447	193.383	125.026	72.459	36.160
9	9	9	20	0.5	391.664	290.032	204.100	133.911	79.607	41.973

Table 1: 250 numerical experiments.

S	K_M^Q	K_M^A	k_1^A	k_{-1}^A	k_2
3:0.1:19	0.996345	0.999979	0.3052	0.1052	0.2000
3:0.5:19	0.996243	0.999978	0.3055	0.1055	0.2000
3:1.0:19	0.996112	0.999977	0.3057	0.1057	0.2000
3:2.0:19	0.995837	0.999975	0.3063	0.1063	0.2000
3:4.0:19	0.995253	0.999972	0.3073	0.1072	0.2000
3:8.0:19	0.994059	0.999972	0.3083	0.1083	0.2000
3:16 :19	0.991973	0.999978	0.3088	0.1088	0.2000
10:0.1:19	0.998506	0.999996	0.3017	0.1017	0.2000
10:0.5:19	0.998495	0.999996	0.3017	0.1017	0.2000
10:1.0:19	0.998481	0.999996	0.3018	0.1018	0.2000
10:2.0:19	0.998374	0.999995	0.3019	0.1019	0.2000
10:4.0:19	0.998320	0.999995	0.3019	0.1019	0.2000
10:8.0:19	0.998215	0.999996	0.3020	0.1020	0.2000
16:0.1:19	0.999024	0.999998	0.3011	0.1011	0.2000
16:0.5:19	0.999022	0.999998	0.3011	0.1011	0.2000
16:1.0:19	0.999020	0.999998	0.3011	0.1011	0.2000
16:2.0:19	0.998967	0.999998	0.3011	0.1011	0.2000

Table 2: Rate constants estimated by $A(S, E) = 0$ or $Q(S, E) = 0$. The first column indicates the measured concentrations of the substrate during the reaction process, and the corresponding concentrations of enzyme is determined by S , so we do not show them explicitly. $a : h : b$ means that the concentrations are measured from $S = a$ to $S = b$ with step length h . K_M^Q denotes the Michaelis constant K_M estimated by $Q(S, E) = 0$, K_M^A denotes the K_M estimated by $A(S, E) = 0$, k_1^A and k_{-1}^A denote k_1 and k_{-1} estimated by $A(S, E) = 0$, if k_2 is provided. Here, we assume that $k_2 = 0.2000$ is exactly estimated by equation (35).